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Serotonin and Dopamine Have Opposite Effects on Phototaxis in Larvae of the Bryozoan *Bugula neritina*

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Abstract. Adult colonies of the bryozoan *Bugula neritina* release short-term anenteric larvae that initially are strongly photopositive. Over the course of several hours larvae lose their initial photopositivity and either become photonegative or alternate between positive and negative phototaxis. We report that newly released photopositive larvae rapidly become photonegative upon exposure to 10^{-6}–10^{-5} *M* serotonin or its metabolic precursor, 5-hydroxytryptophan. This behavior was not observed in two congeners of *B. neritina*, nor in larvae of three other species of bryozoans and seven species from four additional phyla. Antibodies to serotonin label cells in the region of the equatorial nerve-muscle ring and in two tracts extending from the apical disc to this ring. In a separate series of experiments, larvae treated with dopamine (10^{-7}–10^{-5} *M*) significantly prolonged their photopositive period. This effect was also obtained with the D_2 dopamine receptor agonist, quinpirole (10^{-6}–10^{-5} *M*). HPLC analysis determined that newly released photopositive larvae contained 0.120 pmol dopamine/μg protein. These findings implicate serotonin and dopamine as important neurochemical regulators of phototaxis in larvae of *B. neritina*.

Introduction

Regulation of the vertical distributions of pelagic larvae of marine invertebrates is most likely restricted to the vectoral factors of current velocity, light direction, and gravity (Crisp, 1984). Of these, most is known about the influence of light. Studies of the role of phototaxis in larval behavior were pioneered by Thorson (1964), who documented the photic responses exhibited by larvae of 141 species of shallow-water benthic marine invertebrates from 11 phyla. In Thorson's survey, the most frequently observed response to light was for larvae to be initially positively phototactic (82%). Only 6% responded negatively to light throughout larval life, and only 12% were indifferent to light. In 76% of the cases examined, larvae that were initially photopositive became photonegative before the end of the larval period. Apparently, an ontogenetically regulated switch governing the sign of phototaxis is found in larvae of many species. Positive phototaxis early in the larval period may bring larvae up into the water column and facilitate dispersal; later negative phototaxis may bring them down to the substratum where settlement occurs. In addition to changes in phototaxis during development, environmental signals may influence phototactic behavior of larvae (see Crisp, 1984; Young and Chia, 1987, for reviews). Factors such as temperature, salinity, light intensity, ionic shock, pH, exposure to chemical cues from settlement-specific substrates, and pollutants all have been implicated in environmentally induced changes in phototaxis.

Despite the wide phylogenetic distribution and ecological significance of larval phototaxis, very little is known about the internal mechanisms responsible for generating and modulating this behavior. This lack of knowledge stands in contrast to current understanding of the neural bases of stereotyped behaviors in many adult invertebrates. Although that literature is far too extensive

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Abbreviations: 5HT, serotonin; 5HTP, 5-hydroxytryptophan; DA, dopamine; DHBA, 3,4-dihydroxybenzylamine; DDW, deionized distilled water; HPLC, high-pressure liquid chromatography; MBL-ASW, MBL artificial seawater; MPB, Millonig's phosphate buffer; PBS+, phosphate-buffered saline containing 0.1% Triton X-100 and 0.1% NaN_3.

to review here, it is important to point out a common theme that has emerged in the last two decades: Many behaviors are initiated, maintained, altered, or terminated by the action of monoamine or peptide modulators on neural networks (see reviews by Harris-Warrick and Marder, 1991; Katz, 1995). Examples involving monoamines include initiation of swimming in an annelid (Willard, 1981; Mangan *et al.*, 1994); modulation of phototaxis, swimming, respiration, and feeding in gastropods (Crow and Forrester, 1986; McClellan *et al.*, 1994; Syed *et al.*, 1990; Wieland and Gelperin, 1983; Kyriakides and McCrohan, 1989); starting and stopping flight in an insect (Claassen and Kammer, 1985); and switching of body postures and modulation of pyloric motor output in decapod crustaceans (Livingstone *et al.*, 1980; Flamm and Harris-Warrick, 1986).

Although evidence has been presented for the existence of neuroactive monoamines in the larvae of many marine invertebrates—including hydrozoans (McCauley, 1995; Walther *et al.*, 1996), a nemertean (Hay-Schmidt, 1990a), a polychaete (Hay-Schmidt, 1995), bivalve and gastropod molluscs (Coon and Bonar, 1986; Goldberg and Kater, 1989; Marois and Carew, 1990; Barlow and Truman, 1992; Pires *et al.*, 1992), several echinoderms (Toneby, 1980; Burke, 1983; Burke *et al.*, 1986; Bisgrove and Burke, 1986, 1987; Nakajima, 1987, 1988; Thorndyke *et al.*, 1992), brachiopods (Hay-Schmidt, 1992), phoronids (Hay-Schmidt, 1990b,c), a hemichordate (Dautov and Nezlin, 1992), and a cephalochordate (Holland and Holland, 1993)—less is known of the roles of these compounds in the mediation or modulation of larval behaviors. Monoamines have been implicated in the control of metamorphosis in a variety of taxa including hydrozoans (McCauley, 1995; Walther *et al.*, 1996), polychaetes (Biggers and Laufer, 1992; Okamoto *et al.*, 1995), gastropods (Couper and Leise, 1996; Pires *et al.*, 1995), bivalves (Coon and Bonar, 1987; Bonar *et al.*, 1990; Chevolot *et al.*, 1991; Kingzett *et al.*, 1990), a barnacle (Yamamoto *et al.*, 1996), and an echinoid (Burke, 1983). Effects of monoamines on ciliary locomotion have also been reported. Bath-applied dopamine (DA) induces ciliary reversal and backward swimming in some echinoid plutei (Lacalli and Gilmour, 1990; Mogami *et al.*, 1992), while serotonin (5HT) increases the speed of forward swimming (Mogami *et al.*, 1992). Serotonin also accelerates ciliary beat frequency in encapsulated embryos of the gastropod *Helisoma trivolvis*, while DA has no effect (Diefenbach *et al.*, 1991; Goldberg *et al.*, 1994). However, no data have been published on the regulation of larval phototaxis by monoamines or any other neuroactive substances.

Bryozoans are excellent material for experimental studies of larval phototaxis because most species retain their early developmental stages and, on stimulus of light, release larvae that generally settle within a few hours of eclosion. Cohorts of larvae all released within a few minutes of each other can thus be obtained. Ryland (1976, 1977) reviewed in detail the early studies of the phototactic behavior of bryozoan larvae, and several important conclusions come from these studies. First, a range of responses to light are possible, depending on the species. During the course of its larval existence an individual could remain neutral to light throughout; react positively throughout; change from positive to negative or positive to partial negative; and change from positive to alternation between positive and negative (Ryland, 1960). Second, in larvae of *Bugula flabellata* and *B. turrita*, it is possible to artificially force a switch in photic response with agents such as elevated pH, hypotonic seawater, or CuCl (Lynch, 1947). Third, in larvae of *Cryptosula pallassiana*, a rise in temperature decreases the photopositive phase, but the amount of illumination has no effect on the rate of change in sign of the response to light (Ryland, 1962). Furthermore, Ryland observed that pipetting larvae also results in an immediate change from positive to negative phototaxis.

Larvae of many species with phototactic responses possess pigmented epidermal structures that, on the basis of anatomical criteria, are assumed to be photoreceptors (Ryland, 1976, 1977). Not all species with larvae that orient to light have pigmented "eyespots," however (Ryland, 1960); in these species, other specialized epidermal cell types are hypothesized to be photoreceptors (Zimmer and Woollacott, 1989). To date, all pigmented ocelli of bryozoan larvae examined at the ultrastructural level have been based on a sensory cell with an elaboration of multiple cilia that are thought to be the receptoral organelles (Woollacott and Zimmer, 1972; Hughes and Woollacott, 1978, 1980; Reed *et al.*, 1988).

We selected *Bugula neritina* for detailed investigation of the monoaminergic control of phototaxis in the larvae of marine bryozoans. *B. neritina* is cosmopolitan in temperate to tropical waters and is often abundant in specific locales at certain times of the year. Larvae of *B. neritina* are barrel-shaped and about 300 μm in diameter. General features of larval anatomy and events in metamorphosis are well documented for this species (Woollacott and Zimmer, 1971, 1972, 1978; Reed and Woollacott, 1982, 1983). The motive force for swimming is provided by the coordinated beating of the cilia of some 300 elongate coronal cells that form the extensive lateral surface of the larva. Two pigmented "eyespots" are situated on the posterior surface, and these connect with a neural plate in the apical disc by way of an underlying equatorial nerve-muscle ring. Larvae are strongly photopositive on release and with time either

change to photoneutral or alternate between photopositive and photonegative.

We report that 5HT and its immediate metabolic precursor, 5-hydroxytryptophan (5HTP), rapidly caused photopositive larvae of *B. neritina* to become photonegative. Antibodies to 5HT label cells with processes that are associated with the apical disc and extend orally to the equatorial nerve-muscle ring. In contrast, we show that DA and the D_2 DA receptor agonist quinpirole prolonged photopositivity in newly released larvae, and we present chromatographic data to confirm the presence of endogenous DA. We also report qualitative observations on the effects of 5HT and DA on the photic behavior of larvae from five other species of bryozoans and from seven species in four additional phyla. These results demonstrate that the responses observed in *B. neritina* to 5HT and DA are generalizable neither to larvae of congeners nor to a broad range of marine invertebrate larvae.

Materials and Methods

Behavior of larvae of Bugula neritina

Sexually mature colonies of *Bugula neritina* (Linnaeus) 1758 were collected in 1993, during February and March, from the sides of floating docks and other submerged objects in the Indian River near the Smithsonian Marine Station at Link Port, Fort Pierce, Florida. Colonies were maintained in 2-gal aquaria provided with aeration. Release of larvae was initiated by exposing colonies to sunlight following an overnight period of dark adaptation.

Phototaxis experiments were conducted in MBL artificial seawater (MBL-ASW, Cavanaugh, 1956) with the salinity adjusted to habitat level (20‰). Phototactic behavior of larvae was assessed in the presence of 5-hydroxytryptamine hydrochloride (serotonin, 5HT, Sigma); 5-hydroxy-L-tryptophan (5HTP, Sigma); 3-hydroxytyramine hydrochloride (dopamine, DA, Sigma); (±)-SKF-38393 (a D_1 DA receptor agonist, Research Biochemicals Inc.); and (−)-quinpirole hydrochloride (a selective D_2 DA receptor agonist, Research Biochemicals Inc.). Twenty milliliters of MBL-ASW served as the control in all experiments. Drugs were dissolved and diluted directly into MBL-ASW. Final working solutions were in 20-ml volumes and contained 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} *M* concentrations of the drug.

Experiments were conducted in glass Stender dishes with a capacity of 30 ml and an inside diameter of 4.5 cm. Larvae were transferred into dishes by pipetting, usually carrying over about 0.2–0.3 ml of Indian River water. It was not feasible to accurately determine the number of larvae being transferred; consequently, dishes contained unequal numbers of larvae. The number of larvae varied from 20 to 78 per dish, but 25 to 35 was the most common range of values. Pipetting did not affect the responses of larvae of *B. neritina* to light; this result is contrary to the observations of Ryland (1962) with larvae of another bryozoan, *Cryptosula pallasiana*. A 4-ft GE F400CW cool white fluorescent lamp was used as the light source. Dishes containing larvae were placed on white paper 3 in. from the light. Photon flux was measured with a LI-COR model LI-1000 light meter equipped with a detector calibrated for air and measuring between 400 and 700 nm. Irradiance measurements were made in air and ranged between 4.76×10^{15} and 5.78×10^{15} photons/s/cm^2 along the length of the tube. The gradient across the dishes was 6.69×10^{15} to 4.88×10^{15} photons/s/cm^2. Temperature remained within 1°C over the course of individual experiments and ranged from 20°C to 23°C over the course of all experiments.

The number of larvae in the half of the dish that was closest to the light was counted at designated time intervals throughout an experiment. Total numbers of larvae were then counted and the data converted to percentages. A repeated-measures ANOVA was used on arcsin-transformed data to determine whether significant differences existed among treatments, and a Fisher probability least significant difference test was used to localize where these differences, if any, resided (Statview 4.0, Abacus Concepts).

Behavior of other marine invertebrate larvae

The phototactic responses of larvae of 12 additional species of marine invertebrates were evaluated qualitatively (Table 1). Some of these studies were conducted at the Museum of Comparative Zoology in Cambridge, Massachusetts, and others at the Kewalo Marine Laboratory in Honolulu, Hawaii. In all cases, 5HT and DA were applied at a concentration of 10^{-5} *M*. As above, 20-ml volumes in 30-ml Stender dishes were used. A combination of fluorescent and natural light was employed, but irradiance was not quantified. Responses of larvae were gauged qualitatively.

Scanning electron microscopy

Larvae of *Bugula neritina* were fixed in 1% OsO_4 in seawater for 30 min, rinsed in distilled water, and dehydrated in a graded series of acetone. Specimens were critical-point dried in CO_2, gold-palladium coated, and examined with an AMR 1000 scanning electron microscope.

Immunohistochemistry

The immunohistochemical protocol was adapted from that of Kempf *et al.* (1987). Larvae were fixed for 1 h at

room temperature in 4% paraformaldehyde containing 0.14 *M* NaCl and 0.2 *M* Millonig's phosphate buffer (MPB). After fixation, larvae were washed twice in MPB (10 min each). Larvae were then washed in deionized distilled water (DDW) and rehydrated through an ethanol series to xylene (30%, 50%, 80%, 95%, 100%, 100%, xylene, 10 min each), then rehydrated through the same series back to DDW. This procedure increases tissue permeability to antibodies. Larvae were then washed twice (10 min each) in 20 m*M* phosphate-buffered saline in 0.15 *M* NaCl, pH 7.3, containing 0.1% Triton X-100 and 0.1% NaN_3 (PBS+). Subsequent steps were carried out on a shaker table at 4°C. Larvae were incubated for 4 h in a blocking medium consisting of 5% heat-inactivated goat serum in PBS+, then incubated overnight in primary antibody solution. This was a 1:325 dilution (in blocking medium) of polyclonal rabbit anti-5HT (Incstar #20080). A batch of larvae was treated overnight with blocking medium instead of primary antibody solution, as a control for specificity of immunofluorescent labeling. All larvae were then washed four times with PBS+ over a 12-h period, and incubated overnight in the secondary antibody solution. Secondary antibodies were goat anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate (Organon-Teknica-Cappel #12121671), diluted 1:300 in blocking medium. Following treatment with secondary antibody, larvae were again washed four times with PBS+ over a 12-h period, then mounted in a Tris-buffered (pH 9.5) glycerol medium containing 4% *n*-propyl gallate (Giloh and Sedat, 1982). Larvae were examined and photographed under UV epi-illumination.

Chromatographic analysis of dopamine

DA content of larvae was analyzed by high-pressure liquid chromatography (HPLC) with electrochemical detection. Homogenization of larvae and extraction of catecholamines differed only in a few details from the methods of Coon and Bonar (1986). Approximately 75 μl of packed larvae was concentrated by gentle centrifugation and homogenized in a glass tissue grinder on ice in 1.0 ml of 0.4 *N* perchloric acid with 4 m*M* reduced glutathione, 4.7 m*M* EGTA, and 100 n*M* 3,4-dihydroxybenzylamine (DHBA, Sigma) as an internal standard. The homogenate was centrifuged at 15,000 × *g* for 5 min. Two 400-μl aliquots were transferred to 10 × 75 mm glass tubes for extraction of catecholamines over alumina (Anton and Sayre, 1962). To each tube was added 600 μl DDW, 50 mg alumina, and 1 ml extraction buffer (1.5 *M* Tris plus 50 m*M* Na_2 EDTA, pH 8.6). Tubes were agitated 10 min by inversion. Alumina was washed twice with DDW, transferred to microcentrifuge filter assemblies (Rainin #39-402), and catechols were eluted with 200 μl 0.1 *N* perchloric acid. This extract was injected directly into the HPLC system (standard injection volume 80 μl). Each extraction therefore provided enough material for two full sample injections; the remaining material from each extraction was used in smaller injections to help confirm the DA peak while varying the organic content of the mobile phase and "spiking" the chromatographic standards mix (see below). The recovery efficiency of the DHBA internal standard (64%–68%) was calculated for each extraction and used to correct the endogenous catecholamine data from that extraction.

Separation of catecholamines was accomplished by HPLC with an Alltech #28922 Adsorbosphere catecholamine column (100 × 4.6 mm, 3 μm C-18 reverse phase packing). The aqueous portion of the mobile phase contained 100 m*M* monochloroacetic acid, 1.3 m*M* Na_2 EDTA, and 1.3 m*M* sodium octyl sulfate adjusted to pH 3.00–3.05 with NaOH (85 m*M* final concentration). To this was added 1%–5% (v/v) acetonitrile. Flow rate was set at 1.0 ml/min.

Catecholamines were detected with an EG & G/Princeton Applied Research #400 amperometric electrochemical detector with glassy carbon working electrode set at an oxidizing potential of 700 mV against a Ag/AgCl reference electrode (Riggin and Kissinger, 1977; Krstulovic, 1982). Oxidation current range was set at 10 or 20 nA full-scale for the smaller peaks in the first part of the chromatogram, and at 50 nA for DA, which eluted later and was present at higher concentration. Detector output was low-pass filtered (1.0-s time constant) and sent to a Beckman #427 integrator which printed chromatograms and calculated peak areas and retention times.

The DA chromatogram peak was identified by comparing its retention time to that of authentic DA at various concentrations of acetonitrile (1%–5%). A standard curve of DA peak area as a function of amount injected was used to quantify DA concentration in larval homogenates. Reported values of larval DA are derived from the mean of two 80-μl injections, one from each extraction, run at the acetonitrile concentration (5%) that yielded best resolution of the DA peak. Dopamine concentration was expressed as picomoles of free base per microgram of protein. Protein content of the homogenate was assayed by the Coomassie blue dye-binding method (Bradford, 1976) with bovine serum albumin as the standard (Bio-Rad kit, #500-0002).

Results

Monoamine modulation of phototactic behavior of larvae of Bugula neritina

Preliminary experiments conducted during June 1992 in Honolulu, Hawaii, established in a qualitative fashion

that the addition of exogenous 5HT at 10^{-5} *M* concentration brought about an immediate transformation from positive to negative phototaxis in newly released larvae of *B. neritina*. At that time, stocks of adult colonies were insufficient to provide the quantities of larvae needed for a full experimental analysis, but the response was reconfirmed in Hawaiian *B. neritina* during May 1993.

The effects of 5HT and, subsequently, DA on phototaxis were evaluated in detail in February and March 1993 in Fort Pierce, Florida, where a large supply of sexually mature colonies was available.

The effect of 5HT on the percentage of positively phototactic larvae was evaluated at concentrations of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} *M* over a 140-min period (Fig. 1A). In controls without applied 5HT, the percentage of larvae remaining photopositive decreased from a mean of 88% to 70% over the 140-min period. There was no significant difference from the control in experimental treatments with 10^{-8} *M* exogenous 5HT. However, the decrease in photopositivity seen after 140 min in larvae treated with 10^{-7}, 10^{-6}, and 10^{-5} *M* 5HT was much greater than that seen in controls ($P < 0.05$ for 10^{-7} *M*; $P < 0.02$ for 10^{-6} and 10^{-5} *M* 5HT). The change from photopositive was especially rapid at 10^{-6} and 10^{-5} *M* concentrations. At these concentrations, most larvae switched to photonegative within about 1 min after the addition of 5HT; in every trial at 10^{-5} *M* all larvae were photonegative after 110 min. Parallel studies of change in sign of phototaxis were conducted using 5HTP, the immediate metabolic precursor of 5HT (Fig. 1B). Results of these studies documented a shift in phototaxis even more dramatic than that observed with 5HT. The decrease in percentage of positively phototactic larvae after 140 min was significantly greater at 10^{-5}, 10^{-6}, and 10^{-7} *M* 5HTP than in control trials ($P < 0.02$). The response to 5HTP was also more rapid at the higher concentrations than was the response to 5HT at those same concentrations.

The effect of DA on the percentage of positively phototactic larvae was assessed at 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} *M* concentrations over a 240-min period (Fig. 2A). In controls lacking exogenous DA, the percentage of larvae remaining photopositive decreased from a mean of 83% to 41% over 240 min. The addition of DA at 10^{-7}, 10^{-6}, and 10^{-5} *M* levels caused more larvae to remain photopositive than in the untreated controls ($P < 0.02$). Larvae treated with 10^{-5} *M* DA did not show any appreciable loss of positive phototaxis up to 240 min after application of DA. The response of larvae of *B. neritina* to DA was qualitatively reconfirmed using Hawaiian material in May 1993. Application of the D_1 DA receptor agonist SKF-38393 had no significant effect on phototactic

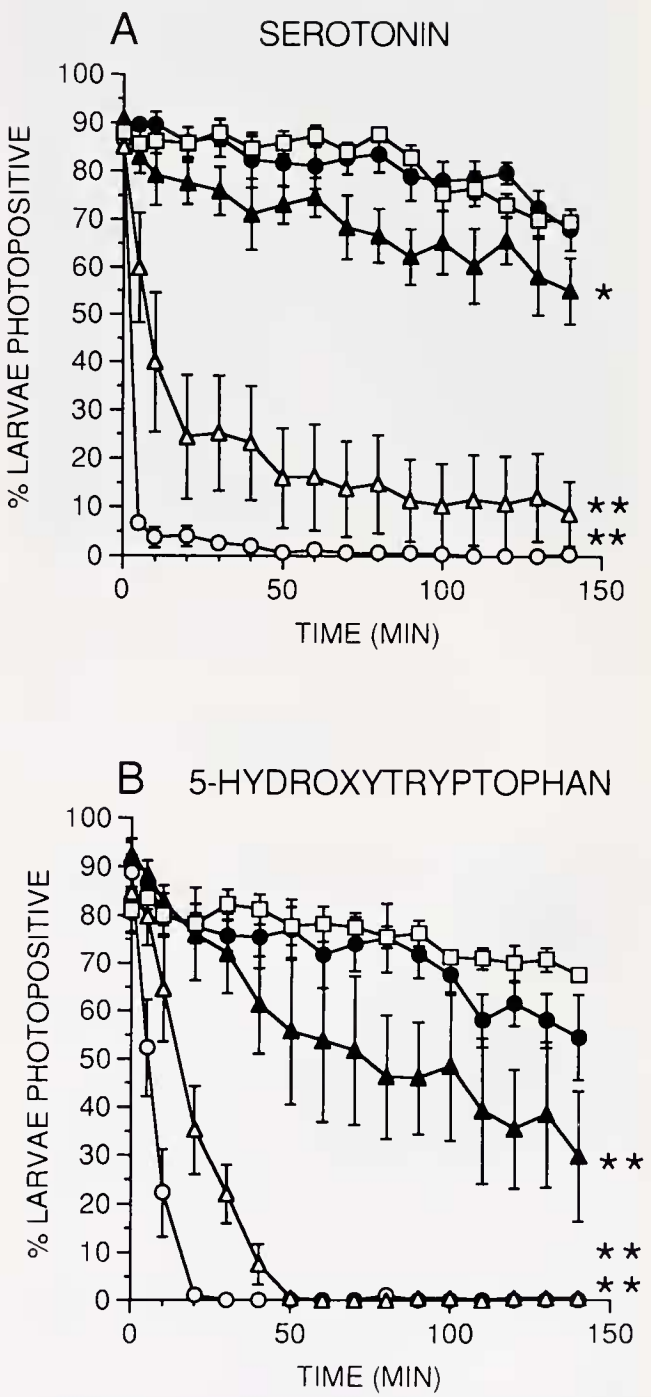


Figure 1. Percentage of larvae of *Bugula neritina* that were photopositive after timed exposure to varying concentrations of (A) serotonin and (B) 5-hydroxytryptophan. Each point represents the mean (±1 SEM) of 5 (A) or 4 (B) replicate trials. Open circles, 10^{-5} *M*; open triangles, 10^{-6} *M*; filled triangles, 10^{-7} *M*, filled circles, 10^{-8} *M*; squares, control. Single and double asterisks indicate significant differences from control at $P < 0.05$ and $P < 0.02$.

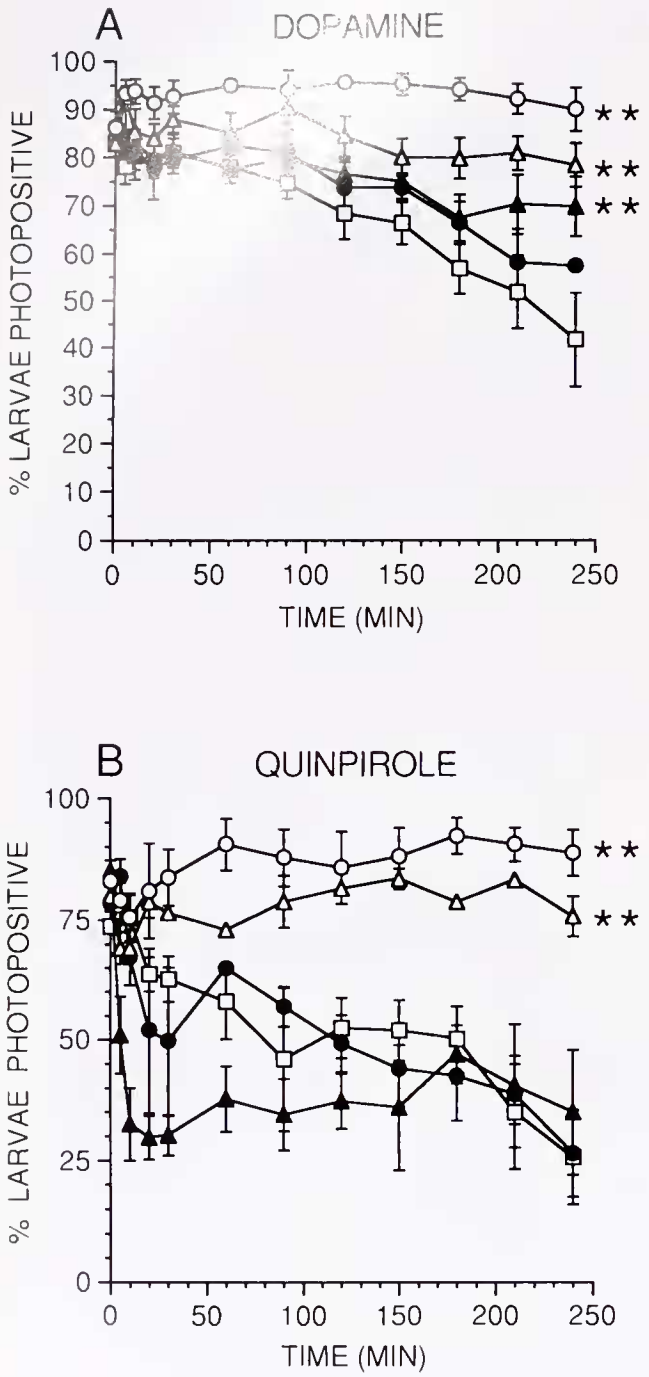


Figure 2. Percentage of larvae of *Bugula neritina* that were photopositive after timed exposure to varying concentrations of (A) dopamine and (B) quinpirole. Each point represents the mean (±1 SEM) of 3 replicate trials. Explanation of symbols as in Figure 1.

behavior of larvae of *B. neritina* at concentrations of 10^{-5} to 10^{-8} *M*, but was clearly toxic to the larvae. The D_2 DA receptor agonist quinpirole, however, mimicked the effect of DA in prolonging positive phototaxis. The percentage of photopositive larvae was significantly greater after 240 min in quinpirole treatments of 10^{-5} and 10^{-6} *M* than in the untreated controls ($P < 0.02$, Fig. 2B). Application of quinpirole at 10^{-7} and 10^{-8} *M* produced an initial decrease in the percentage of photopositive larvae compared with the controls, but the difference was no longer significant after 240 min.

Localization of serotonin

Larvae of *B. neritina* have two pigmented eyespots situated in two depressions on the posterior lateral surface of the larva (Fig. 3). The lateral surfaces of the larva are formed by heavily ciliated cells of the corona, the larval locomotory organ. An apical disc complex with a central neural plate is situated at the aboral pole of the larva.

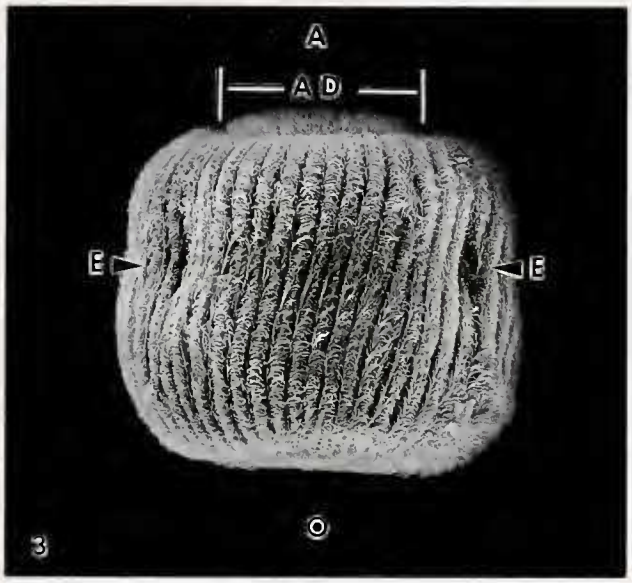
Immunolocalization of 5HT was achieved in larvae of *B. neritina*, but the procedure resulted in distortion of larvae and made precise determination of sites of the reaction difficult (Fig. 4). Strong 5HT-like immunoreactivity is evident, however, in the region of the equatorial nerve-muscle ring and in two tracts extending from the apical disc to this ring. These tracts are in a position occupied by a nerve-muscle tract extending from the roof of the metasomal sac and equatorial nerve-muscle ring to the neural plate in the center of the apical disc. The equatorial nerve-muscle ring underlies the two posterior lateral pigmented eyespots. No fluorescence above background was observed in control larvae in which the anti-5HT primary antibody was omitted.

Chromatographic analysis of dopamine

Reversed-phase HPLC of two alumina extracts of a homogenate of newly released larvae of *B. neritina* yielded chromatograms that included several peaks, including one that was identified as DA on the basis of its co-elution with authentic DA over a range of mobile-phase acetonitrile concentrations (Fig. 5). The two extractions resulted in DA values of 516 and 481 pmol/homogenate; these were averaged and divided by the total soluble protein content of the homogenate to yield a final estimate of larval DA content of 0.120 pmol/μg protein. Dihydroxyphenylalanine and norepinephrine may also be present in larvae of *B. neritina* (Fig. 5): all chromatograms showed at least a partially resolved peak corresponding to each of these monoamines, but we did not have enough material to justify reporting quantitative values.

Comparative analysis of larval phototaxis

We examined the effects of 5HT at 10^{-5} *M* concentration, and in some cases 10^{-5} *M* DA as well, on phototaxis



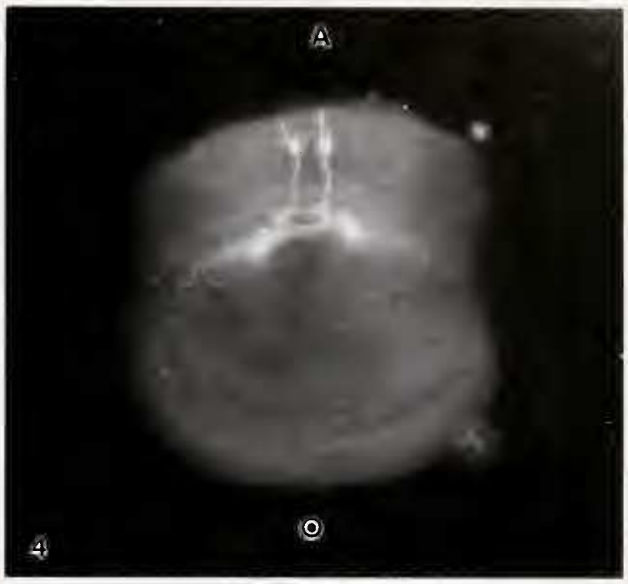


Figure 3. Scanning electron micrograph of *Bugula neritina* larva taken from lateral view. The metachronal waves of cilia mark the locations of the strap-like elongated coronal cells that together form the larval locomotory organ. Depressions in which the two pigmented eyespots (E) are situated are visible on the posterior lateral surface. The opening of the metasomal sac marks the oral pole (O) of the larva, and the ciliated apical disc (AD) is located at the aboral pole (A). ×200

Figure 4. Light photomicrograph of 5HT-like immunoreactivity in larva of *Bugula neritina*, with orientation of larva positioned to match that in Figure 3. Fluorescence is associated with position of equatorial nerve-muscle tract that underlies eyespots and is continuous with two fluorescent tracts that extend into apical disc. ×200

of larvae from a number of species (Table I). Although the analysis was based exclusively on a qualitative evaluation of responses to the addition of these monoamines, we were unable to document a pattern similar to that observed in *B. neritina* in larvae from two congeners and three additional species of bryozoans. In a broader survey we did not detect any effects of monoamines on phototaxis of larvae of one copepod crustacean, one gastropod, two demosponges, and two ascidians. The larvae of the gastropod *Phestilla sibogae*, however, did present increased motility and greater positive phototaxis when exposed to 10^{-5} *M* 5HT.

Discussion

Bath-application of 10^{-5} *M* or 10^{-6} *M* 5HT rapidly induced negative phototaxis in newly released photopositive larvae of *B. neritina* (Fig. 1A). A similar response was obtained with the same concentrations of 5HTP, the immediate metabolic precursor of 5HT, although the onset of negative phototaxis took about 10–15 min longer with 5HTP than with 5HT (Fig. 1B). This result is consistent with the notion that serotonergic cells may take up exogenous 5HTP and convert it to 5HT, thus augmenting releasable endogenous stores of that neuromodulator. In the gastropod *Lymnaea stagnalis*, injection of 5HT activates rhythmic shell movements; the same motor program can be obtained by injection of 5HTP, which also increases levels of 5HT and firing of serotonergic neurons (Kabotyanski *et al.*, 1992).

Dopamine, when bath-applied in concentrations from 10^{-7} *M* to 10^{-5} *M*, prolonged the initial period of photopositivity in larvae of *B. neritina* (Fig. 2A). This effect was mimicked by the mammalian D_2 DA receptor agonist quinpirole (Fig. 2B) at 10^{-6} *M* and 10^{-5} *M*. The sharp initial decrease in the percentage of photopositive larvae seen after treatment with 10^{-7} *M* quinpirole, contrasted with enhancement of phototaxis at higher concentrations, may reflect interactions with more than one class of DA receptor; no pharmacological profiles of bryozoan DA receptors are available.

Under normal laboratory conditions, larvae of *B. neritina* remain photopositive for 2–3 h (see controls in Fig. 2), then make a transition to a state in which they alternate between positive and negative phototaxis (Ryland, 1960), and eventually may become photonegative (Mawatari, 1951). Lynch (1947) never observed negative phototaxis in this species under normal laboratory conditions of lighting, temperature, and salinity, but he was able to induce larvae to become photonegative by

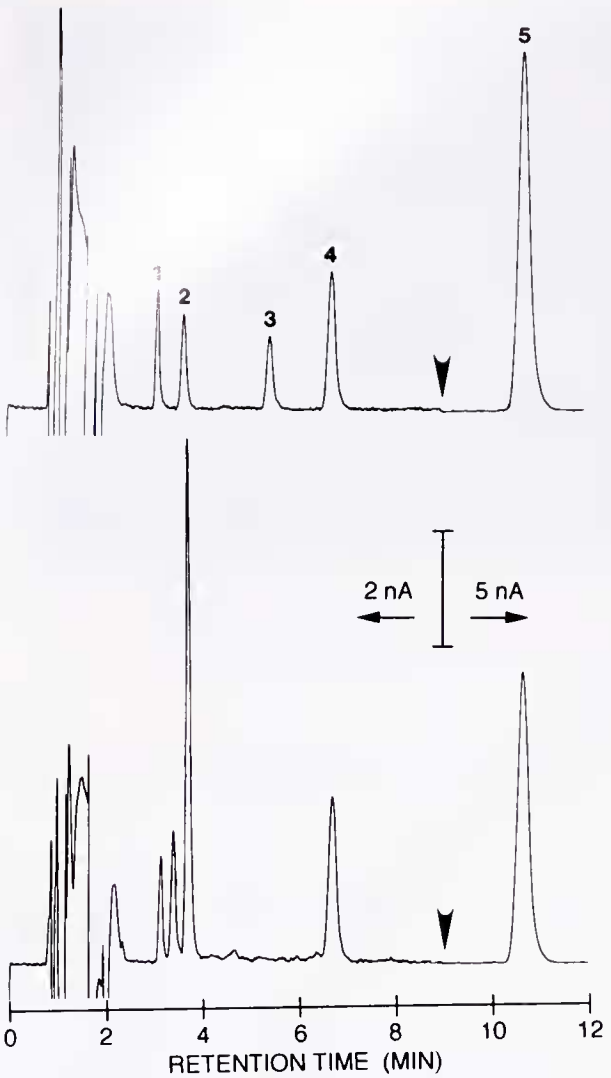


Figure 5. Separation of catecholamines by HPLC with electrochemical detection. Upper trace: chromatogram of catecholamine standards mixture (3.2 pmol each of [1] dihydroxyphenylalanine, [2] norepinephrine, [3] epinephrine; 6.4 pmol [4] DHBA; 64 pmol [5] DA). Lower trace: chromatogram of alumina extract of homogenate of newly released larvae of *Bugula neritina*. Current scale changes at time marked by vertical arrows.

exposing them to intense light. He inferred from distributions of adult colonies in the field that most larvae in nature are probably photonegative at the time of settlement and metamorphosis.

It is possible that 5HT and DA are neurochemical effectors of an ontogenetic switch in the sign of phototaxis, opposing peripheral cilioexcitatory and both peripheral and central cilioinhibitory roles, respectively of serotonin and dopamine, have been described in the gill epithelium of the bivalve *Mytilus edulis* (Catapane *et al.*, 1978; [illegible] *et al.*, 1986). However, such mechanisms are probably not adequate to explain the aminergic control of cilia-driven phototaxis documented in this report. Because 5HT and DA exert opposite effects on the sign of phototaxis when applied homogeneously to the entire animal, it seems more likely that they modulate the interaction between photoreceptor organs and the ciliated coronal cells that are the effectors of phototaxis (Woollacott and Zimmer, 1972). Larvae of *B. neritina* always swim with the aboral pole directed forward, while rotating clockwise about the oral-aboral axis. In both positive and negative phototaxis the sensory feedback to the larva's locomotion should operate to equalize the light input to the two laterally situated photoreceptor organs; this would keep the oral-aboral axis parallel to the direction of the light stimulus. The crucial difference between positive and negative phototaxis is expected to be in the larva's course-correction mechanism. In positive phototaxis, asymmetric illumination of the two photoreceptor organs should result in inhibition of cilia on the more-illuminated side, or excitation of cilia on the less-illuminated side (Woollacott and Zimmer, 1972). To achieve negative phototaxis, the opposite course-correction strategy would be required: inhibition of cilia on the less-illuminated side, or excitation of cilia on the more-illuminated side.

If phototaxis is synaptically mediated by cells of the equatorial nerve-muscle ring, as has been suggested on anatomical grounds (Woollacott and Zimmer, 1972), one can propose that a functional "rewiring" of the phototaxis control system might be accomplished by monoamine neuromodulators. That is, whether a light stimulus to a photoreceptor organ excites or inhibits a given population of cilia might depend on the neuromodulatory milieu of the synapses in the phototaxis control system. In the pyloric network of the stomatogastric ganglion of the lobster *Panulirus interruptus*, the qualitative pattern of functional connections between identified neurons has been shown to depend critically upon the modulatory neurohormonal environment of the circuit (Johnson *et al.*, 1995). In two instances DA actually reverses the sign of an identified mixed chemical/electrical synaptic connection, enhancing chemical inhibition and reducing electrical coupling so that the net synaptic interaction changes from excitatory to inhibitory (Johnson *et al.*, 1993).

Although we have demonstrated clear and dramatic effects of bath-applied 5HT and DA on the photic behavior of larvae of *B. neritina*, participation of endogenous 5HT and DA in the control of phototaxis remains to be established. However, such roles for these amines seem quite likely. Processes of 5HT-immunoreactive cells are localized in the equatorial nerve-muscle ring (Fig. 4), where they are situated to modulate the interactions be-

Table I

Change in phototactic swimming behavior of initially photopositive larvae to exogenously applied monoamines (10^{-5} M)

Phylum	Species	Larval type	Phototaxis examined	Monoamine	Response	Source	Date
Arthropoda	*Acartia tonsa*	Nauplius	Positive	5HT	None	North Atlantic	11/93
Bryozoa	*Amathia distans*	Coronate	Positive	5HT	None	Honolulu, HI	5/93
				DA	None		
	Bugula neritina	Coronate	Positive	5HT	Abrupt switch to negative	Honolulu, HI	6/92
					Abrupt switch to negative	Fort Pierce, FL	2/93
					Abrupt switch to negative	Honolulu, HI	5/93
				DA	Slows switch to negative	Fort Pierce, FL	2/93
					Slows switch to negative	Honolulu, HI	5/93
	Bugula stolonifera	Coronate	Positive	5HT	None	Woods Hole, MA	9/93
	Bulgula turrita	Coronate	Positive	5HT	None	Woods Hole, MA	9/93
	Hippopodina feegeensis	Coronate	Positive	5HT	None	Honolulu, HI	5/93
				DA	None		
	Schizoporella sp.	Coronate	Positive	5HT	None	Honolulu, HI	5/93
Mollusca	*Crepidula fornicata*	Veliger	Neutral	5HT	None	Woods Hole, MA	11/93
				DA	None		
	Phestilla sibogae	Veliger	Positive	5HT	>motility, >positive taxis	Honolulu, HI	5/93
				DA	None		
Porifera	*Aplysilla* sp.	Parenchymella	Positive	5HT	None	Honolulu, HI	8/94
	Halichondria coerulea	Parenchymella	Neutral	5HT	None	Honolulu, HI	8/94
Urochordata	*Ascidia ceratodes*	Tadpole	Positive	5HT	None	Monterey, CA	11/93
				DA	None		
	Ciona intestinalis	Tadpole	Positive	5HT	None	Boston, MA	11/93
				DA	None		

tween the putative photoreceptoral organs and the coronal effectors of locomotion (Woollacott and Zimmer, 1972). Dopamine was not localized in the present study, but amounts of DA per microgram of total protein in newly released larvae of *B. neritina* are within the range reported for molluscan larvae, in which DA has been implicated in the control of settlement (Coon and Bonar, 1986; Bonar *et al.*, 1990) and metamorphosis (Pires *et al.*, 1992; Pires *et al.*, 1995).

It is indeed puzzling that newly released photopositive larvae of *B. stolonifera* and *B. turrita* were unresponsive to 10^{-5} *M* bath-applied 5HT. It may be that in these species the conditions that would permit 5HT to influence phototaxis do not apply early in larval life, or at all. Resolution of this issue will require detailed neurochemical and behavioral investigations across the duration of the larval period.

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